AN EARLY GROWTH STUDY ON POSSIBLE PHOTOSYNTHETIC VARIANCES IN TRANSGENIC PLANTS:

A FOURTH YEAR STUDY

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ABSTRACT

Faced with a geometrically increasing world population, agriculturalists have turned to genetic engineering to revolutionize production, which has led to conflicting opinions on the possible effects of these genetic alterations. While there is much support for the many benefits, prevalent opinion among the non-agricultural community seems to be that genetic engineering will have a far-reaching, detrimental impact. Given all the controversy juxtaposed against the numerous benefits, the author decided to study the subject further.

A review of literature pertaining to genetic engineering was conducted. The review determined that the implications of genetically altering plants are not known. Critics of biotechnology claimed that genetic engineering would cause unwanted changes in plants, making them unsafe. Based on the review of literature, the hypothesis formulated for the study was that the rate of photosynthesis is the same in genetically altered plants and non-genetically altered plants.

Two varieties of cotton - Round-Up Ready and standard, non-transgenic - were grown by the researcher in his high school greenhouse. These were used in a comparison to test for differences in photosynthesis rates. The standard variety served as a control group and the Round-Up Ready variety served as the experimental group. The experiment was conducted at a local USDA Agricultural Research Station. A flourometer was used to measure fluorescence; after dark acclimating the plants, they were irradiated with a burst of light. Fluorescence was evaluated because it can be used as a measure of photosynthesis rates. A spreadsheet was designed to record and analyze the rates of fluorescence in this study. The experiment resulted in a finding that the rates of photosynthesis for both the cotton varieties were similar with no significant differences. Therefore, the hypothesis was accepted.

Since the fluorescence rates were similar, the conclusion was that genetically engineered plants are probably safer than some critics believe. Implications were that consumers need to understand that genetic engineering is just a natural progression of methods as agriculturists seek to produce food inexpensively. Recommendations called for a more in-depth study of the differences in standard and genetically engineered plants to help validate this experimental research. It was further recommended that the results of such research be the center point of a public relations campaign by the agriculture industry to educate consumers about the safety of genetically engineered agricultural products. Recommendations closed with a description of the need for more middle school agriculture programs.

INTRODUCTION

Since the beginning of time, people have struggled daily simply to feed themselves. The discovery of agricultural methods around 8000 B.C. (Agriculture, 2001) eased this struggle somewhat. Following this discovery has been an effort to find more efficient ways to produce food. The focus on higher crop yields was passed initially from generation to generation. Now research scientists and farmers alike carry the torch to ensure greater yields using more efficient production methods. This increased production has been sought after in many ways. Examples would include irrigation, the invention of the plow, crop rotation, and more recent agricultural inventions.

Problem Statement

Recently, agriculturists have been directly altering the genetic structure of plants in an effort to improve crop production. This restructuring is conducted by introducing a new gene into the plant. For example, one of these is the gene that produces Bacillus thurigiensis, or BT, a protein that is commonly used in corn to protect it from certain types of caterpillars (Thompson, 2000). Another gene that is used is one that makes a crop resistant to certain types of herbicides that are used to kill weeds. These and other desirable traits have been introduced by genetic engineering. However, the full ramifications of genetic alterations such as these are not yet known. The Food and Drug Administration claims to test to make sure only desirable traits are introduced into the crop, but they have limited time and money to devote to testing (Thompson, 2000).

Many people are concerned with the effects of genetically altering plants (Passacantando, 2000). The problem is that the potential adverse side affects of directly changing the DNA of plants is not yet known. Just like in earlier societies, most food for contemporary society comes from plants. People have the right to know exactly what is being done to the food they eat.

The purpose of this research is to determine if the rate of photosynthesis is different in genetically altered plants than in unaltered or standard plants. If a plant's photosynthesis processes do not function correctly, growth could be severely inhibited. The production of lower quality plants could result, and in turn, reduced crop yields could emerge. Also, if the rate of photosynthesis were different, it would give critics another reason to reject genetically altered crops.

The author has conducted studies related to this project over the past three years (Ruddle; 2001, 2002, 2003) comparing photosynthesis rates of standard and genetically altered plants. More details regarding these projects and other related information can be found in the REVIEW OF LITERATURE.

REVIEW OF LITERATURE

A review of literature from a variety of sources concerning genetics and photosynthesis was conducted to learn more about biotechnology and photosynthesis. Popular agricultural magazines, newspapers, governmental sources, and the Internet were examined for information related to the research. This search revealed information relating to genetically altered crops, photosynthesis, consumer confidence, and related scientific research.

Examples of Genetically Altered Crops

According to the Food and Drug Administration (Thompson, 2000), many crops being grown are already genetically altered. Nearly half of the United States (US) 1999 soybean crop had a gene in it that made it resistant to an herbicide commonly used to control weeds. Also, about a quarter of the corn crop had a gene that produces a toxin that kills caterpillars. Until recently, crops were only genetically altered using selective breeding, the process identified by Mendel. Now people use these modern biotechnology techniques to insert Bacillus thurigiensis and, in other instances, enzymes that make crops herbicide resistant. This process makes it more economical and safer to produce crops. Two popular methods used in plant genetic engineering are utilizing transfer plasmids or a gene gun.

Photosynthesis

According to the University of Arizona (*Physiology* . . ., 1998), photosynthesis means, “ to put together with light.” Plants go through this process using carbon dioxide, water, and light. The plant will die if any of these inputs are removed. Even though all components are important, light is thought to be most important by experts. Photosynthesis seems to operate most efficiently between 65 and 85 degrees F. If the photosynthesis mechanism is affected negatively the plant will probably soon die. If this occurred on a wide scale, it could set off a chain of events that would lead to the downturn of agricultural markets worldwide and possibly cause the downfall of society as extremists fear.

Consumer Confidence

According to a series of articles published in the *Valdosta Daily Times* (October 20, 22, November 5, December 4, 2000), Aventis CropScience was under pressure regarding its StarLink brand of corn. StarLink corn contains BT, a bacterium that protects it from caterpillars. The protein found in it, Cry9C, gives it the Food and Drug Administration rating of not suitable for human consumption according to *Progressive Farmer* (No author, December, 2000) The crop was intended to be used in animal feed. A scare about this corn, caused by information which was later revealed to be incorrect, prompted nationwide recalls of taco shells and many Kellogg's products because some people do not feel safe eating genetically altered corn.

The reason for this concern is because it is believed that some people may be allergic to the Cry9C protein even though no allergies have been proven. The FDA's original belief is that repeated exposure to Cry9C may cause an allergy in humans. But the FDA and the National Center for Environmental Health later published a report (*Investigation* . . ., 2002) that indicated no reason to believe that the Cry9C protein was an allergen in humans. Woznicki (2000) agrees that some people feel that there are many benefits and hazards in genetically altering plants.

Previous Studies

Three years ago the author began researching possible differences in genetically altered and standard crops (Ruddle, 2001). Standard, non-transgenic, soybeans of the DP7331 variety and Round-Up Ready, transgenic, soybeans of the DP6880RR variety were evaluated for differences in photosynthetic rates. They were tested using an Opti Science 500 Modulated Flourometer. This project found little difference in the photosynthesis rates.

Two years ago the author completed a study on a related topic (Ruddle, 2002). Standard soybeans of the DP6926 variety and Round-UP Ready soybeans of the DP6880RR variety were grown and evaluated for differences in photosynthetic rates. One group was tested 21 days after being planted and the other group was tested after 96 days. Paper chromatography measured the essential plant pigments and a spectrophotometer was used to measure DPIP reduction. Some differences were found between the early and late growth groups.

Another study (Ruddle, 2003) focused on Round-Up Ready and standard, non-transgenic, cotton, DP5415 and DP5415RR varieties, and corn, DK687 and DK 687RR varieties. Early and late growth studies were conducted to provide a better overall picture of possible photosynthetic variances. A spectrophotometer was used to measure the reduction of DPIP, which indicates the rate NADP is being raised to $NADPH^+$. No significant difference was found between genetically altered or standard plants in either the early or late growth groups.

Summary

In this search of literature, information was found on modern biotechnology methods of improving plants and photosynthesis was found to be a very important to the growth process. The advantages of genetic engineering and the disadvantages (Parry, 1999) were discussed in the literature. Information on consumer acceptability of genetically altered crops was found, as were negative publicity problems related to genetically altered plants. Finally, scientific research revealed little unwanted differences caused by genetic engineering.

PROCEDURES

Hypothesis

The hypothesis for this project is that genetically altered crops do not have a different rate of photosynthesis than non-genetically engineered crops. The literature detailed no solid evidence that suggests that the rate of photosynthesis is changed. This hypothesis was developed according to the findings in the background study, as detailed in the REVIEW OF LITERATURE section of this report.

Study Design

The hypothesis for this project was tested by using an experimental design. An experimental group of genetically altered plants and a control group of standard plants were evaluated for factors indicating photosynthetic rates using a photosynthesis meter/personal computer combination. A control group was evaluated in the same manner. The data was recorded and analyzed on a spreadsheet to evaluate for any differences and the significance of any variances.

TABLE 1

Materials and Equipment

Item	Quantity
1835 trays	4
4” nursery pots	42
Fafard 3B Soil Mix	1
dibble stick	1
DP5415 seeds	160
DP5415 RR seeds	160
Heating pad	1
Greenhouse	1
Growing bench	1
Watering hose	1

The plants were grown at the Lowndes High School greenhouses. First, the author prepared the plant trays for this early growth study. He prepared four trays that contained 40 two-inch soil pots each**.** Next, the trays were labeled to indicate which variety would be planted in a certain location. After watering, two seeds were then planted one inch deep in each pot, using a dibble stick**.** Three hundred twenty seeds, one hundred sixty of each seed variety, were planted. The soil was then watered again, and the trays were moved to their bench in the greenhouse. Every two or three days the author checked on the plants and watered as needed.

On March 26, 2004, the author went with his FFA advisor to the United States Department of Agriculture Research site in Tifton, Georgia, to test the photosynthesis rates of the plants. The plants were placed in black, opaque bags to dark acclimate the plants prior to arrival at the laboratory. After introductions to lab director, Mrs. Nancy Hand, the researcher went to the lab to test the plants.

First, the calibrations were checked on the Opti Science 500 Modulated Flourometer. The test mode was FV/FM, which is a quantum yield measurement commonly used in photosynthesis experiments. This test mode tells the user the FO, florescence at rest, FM, florescence at the peak of photosynthesis, and the FV/FM quantum yield. The calibrations were as follows: Modulation-49, Saturation-193, Far Red-0, Duration-85, Actinic-0; Detector Gain mode, Temperature Clip-173. The tester then removed a leaf from the opaque bag. The leaf of the plant was then put under the testing area, and the tester pushed the measure button on the machine. The machine irradiated the leaf with an intense burst of light, and then the results appeared on the screen. The researcher recorded the results in his journal to transfer to the data collection spreadsheet for analysis later. This process was repeated until twenty-one samples from each group were tested. The results of the experimentation are detailed in the RESULTS section.

Limitations

This study has several factors that limit its effectiveness. While this project is set up as a true experiment, not enough samples of different species of plants were tested to truly evaluate the differences, if any, among all genetically altered plants. Therefore, a generalization to all genetically altered species and the requisite number of infinite combinations will not be able to be made as a result of this research. Part of this limitation is that author did not have enough funds and time to test enough different plants necessary to make such a broad judgment. According to Andy Harrison (personal communication, December 18, 2000), there are infinite combinations for genetic altering and crossbreeding.

For example, consider the number of possible combinations in the genetic engineering of a plant. One likely scenario is that there are two plants that have desirable characteristics. On average, plants have from ten to twenty chromosomes that govern traits. Consider the rice plant which has twelve chromosomes (*Completion* . . ., 2002). When crossing the wanted characteristics from two plants, the possibility of seventy-eight different combinations of chromosomes exists. Within these twelve chromosomes are over 250 million nucleotide base pairs. A virtually infinite number of different genetic combinations in the world exist when one considers transgenic engineering efforts, in which desirable traits of species are actually crossed. Based on these infinite possibilities, the author would have to plant many different species of plants in large amounts to be able to make a very accurate judgment. The author did not have proper preparation for such a grand experiment.

RESULTS

The data gathered in the experimentation demonstrated several findings. The "FO" number, or dark acclimated, shows the activity of the plant in the dark, prior to irradiation by the intense light. The "FM" number indicates when the plant is at the peak of photosynthesis, immediately following irradiation by the burst of light. The "FV/FM" number or quantum yield is the measurement of the change in the photosynthesis rate from FM to FV. FV is the photosynthesis rate when the plant has returned to normal activity.

Non-Genetically Altered Cotton

Table 2 pertains to the non-genetically altered, standard, cotton that served as a control group in the experiment. This table details the results of the measurements yielded by the experiment.

TABLE 2

Photosynthesis Measurements on Standard Cotton

Plant Number	FO	FM	FV/FM
1	50	86	.418
2	106	230	.539
3	290	330	.121
4	94	195	.517
5	275	336	.181
6	144	278	.482
7	255	351	.273
8	48	87	.448
9	76	128	.406
10	202	310	.348
11	141	262	.461
12	65	166	.608
13	97	205	.526
14	268	370	.275
15	68	140	.514
16	125	182	.313
17	51	86	.466
18	251	331	.241
Averages	144	266	.397
Standard Deviation	85.25	95.40	.132

In Table 2, the range for the "FO" number was 48-275. The range for the "FM" number was 86-370. The range for the "FV/FM" number was .121-.539.

Genetically Altered Cotton

Table 3 pertains to the genetically altered cotton that served as an experimental group in the experiment. This table details the results of the measurements yielded by the experiment.

TABLE 3

Photosynthesis Measurements on Round-Up Ready Cotton

Plant Number	FO	FM	FV/FM
1	252	310	.187
2	121	201	.398
3	118	215	.451
4	118	213	.483
5	68	162	.629
6	44	125	.648
7	83	122	.319
8	94	213	.558
9	141	215	.344
10	297	333	.108
11	176	290	.393
12	87	131	.335
13	80	196	.591
14	143	225	.364
15	131	242	.458
16	89	167	.467
17	268	331	.129
18	106	158	.329
19	75	178	.578
20	67	153	.562
21	70	95	.263
Averages	125	204	.409
Standard Deviation	67.63	66.35	.153

The range for the “FO” number was 44-297. “FO” is the dark acclimated photosynthesis rate. The range for the “FM” number was 95-333. The “FM” number indicates when photosynthesis peaks. The range for the “FV/FM” number was .129-.648. The “FV/FM” number is the difference of the regular and the peak photosynthesis.

Comparison of Altered and Standard Plants

Tables 4 to7 compare and contrast the information gleaned from both the genetically altered, or Round Up Ready, plants and the standard plants. Listed here is information needed to test the project’s hypothesis.

TABLE 4

SUMMARY

Groups	Count	Sum	Average	Variance
FO-Standard	18	2606	144.77778	7696.535948
FO-Transgenic	21	2628	125.14286	4802.128571
FM-Standard	18	4073	226.27778	9637.153595
FM-Transgenic	21	4275	203.57143	4623.057143
FV/FM-Standard	18	7.137	0.3965	0.018558853
FV/FM-Transgenic	21	8.594	0.4092381	0.02460119

TABLE 5

ANOVA-FO

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	3736.676	1	3736.6764	0.609374048	0.439989503	4.105459084
Within Groups	226883.7	37	6131.9914			
Total	230620.4	38				

TABLE 6

ANOVA-FM

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	4997.143	1	4997.1435	0.721418399	0.401141428	4.105459084
Within Groups	256292.8	37	6926.8312			
Total	261289.9	38				

TABLE 7

ANOVA-FV/FM

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.001573	1	0.0015727	0.072058015	0.789854597	4.105459084
Within Groups	0.807524	37	0.021825			
Total	0.809097	38				

The researcher conducted an analysis of variance (ANOVA) test to evaluate for a difference in photosynthesis rates between the Round-Up Ready and standard, non-transgenic cotton. A single factor comparison was used that compared the rates of photosynthesis between the two groups. Since the P-Value for all three sets of data; FO, FM and FV/FM; was above .05, no significant difference in photosynthesis rates existed.

DISCUSSION

Conclusions

The practical considerations of this research include utilization of this model in continued research for improving genetically altered plants. Before researching this subject, the author never thought about the implication of genetic engineering on unrelated basal processes such as photosynthesis. As a result of the experimentation, the author has reached two conclusions. The first conclusion the author reached as a result of the research was that people are still not comfortable with the idea of genetically altered plants. Agriculture companies that utilize biotechnology should have their public relations departments work harder to make consumers aware of the benefits and safety of their products. The second conclusion that the author reached is that, in the earlier stages of growth, the Round-Up Ready cotton is efficient in its photosynthetic processes, as the standard, non-transgenic cotton. This occurrence supported the previously formulated hypothesis.

Implications

Because the author's hypothesis was proven correct, genetically altered plants may not be as dangerous as some groups have thought. This project proves that the genetically altered plants have fewer unwanted side effects than some critics have asserted. After disseminating this information, the agriculture industry can focus on other challenges. This new information could also be an indicator to agricultural companies or other related organizations to conduct similar tests. The findings of this research could be beneficial, especially for generating related studies.

Different companies' public relations departments could publicly release this data to consumers. A media campaign comprised of this data and other helpful information could help lessen the fear of genetically altered crops. Consumers need to understand that the use of genetically engineered foods is just a natural progression of agricultural methods. Experts say that it is simply a speeding up of a selective mutation process (personal communication, Andy Harrison, December 18, 2000).

Recommendations

This study yielded data that was only somewhat useful because of limited sample size, despite the fact that the research was conducted as a true experiment. The author lacked funds, facilities, and full-time researchers to test a broad variety of genetically and non-genetically altered plants that would have made this data more useful. More meaningful data could be realized in a study that is scientifically structured to generate reliable results with an allowed margin of accuracy across a broader sample size. The United States Department of Agriculture's Agriculture Research Service or other similar agencies may be interested in funding such a study.

The original study was performed by the researcher following the year that the county school system administration made the decision to save money and eliminate the middle school agriculture programs at the two county middle schools. This study would have been much easier to conduct if a knowledgeable person such as a teacher of agriculture had been at the author's middle school. Fortunately, the author was able to be advised on the project by a high school agriculture teacher; however, it would have been much easier to contact an advisor at his school to help facilitate this project.

The author would like to close with a recommendation to the Georgia and the US Department of Education to continue its efforts to include more middle school agriculture classes in its agriculture curriculum. Perhaps a state- or nationwide effort would reinstate agriscience in the author's former middle school and many other middle schools, improving agricultural education opportunities for students across the state of Georgia and the United States of America.

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